—Regular Articles—

Effects of Mouth Washing Procedures on Removal of Budesonide Inhaled by Using Turbuhaler

Haruko YOKOYAMA,[a] Yoshikazu YAMAMURA,[b] Takeshi OZEKI,[a] Tatsuji IGA,[c] and Yasuhiko YAMADA*,[a]

[a]*School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432–1 Horinouchi, Hachioji, Tokyo 192–0392, Japan,* [b]*Department of Hospital Pharmacy, Tokyo Postal Services Agency Hospital, 2–14–23 Fujimi, Chiyoda-ku, Tokyo 102-8798, Japan and* [c]*International University of Health and Welfare, 2600–1 Kitakanemaru, Ohtawara, Tochigi 324-8501, Japan*

(Received February 1, 2007; Accepted March 15, 2007)

Mouth washing after inhalation of corticosteroids is effective for prevention of local adverse effects. We determined the amounts of drug residues remaining on the oropharyngeal mucosa following inhalation of budesonide (BUD) via a Turbuhaler (BUD-TH) (100 μg). Further, we studied the effects of mouth washing on the removal of drug residues by quantification of BUD in expectorated wash solution using an HPLC method. The amount of BUD recovered after gargling and rinsing for 5 s each was 19.4 ± 9.4 μg, as compared to 23.8 ± 13.6 μg after rinsing alone for 10 s and 18.3 ± 8.9 μg after gargling alone for 10 s, though the differences were not significant. Our results indicated that about 20% of the dose was remaining on the oropharyngeal mucosa after inhalation. In a comparison of washing times, the amounts of BUD recovered were 26.3 ± 3.2 μg after gargling and rinsing for 3 s each, and 19.4 ± 9.3 μg after those for 5 s each. As for the effect of lag time before beginning mouth washing, the ratio of BUD recovered following mouth washing with a lag time of 1 min was 73.2%, while it was reduced to 27.8% after 10 min, as compared to immediate mouth washing following administration. Our results suggest that the amount of BUD removed by mouth washing is associated with the lag time between inhalation and mouth washing, however, not with the duration of mouth washing. We concluded that immediate mouth washing after inhalation is most useful for the removal of drugs following BUD-TH administration.

Key words——budesonide; immediate mouth washing; mouth washing procedures; prevention; local adverse effects

INTRODUCTION

Inhalation of corticosteroids is recognized as an important first line of anti-inflammatory defense therapy and the drugs have become primary agents in the treatment of asthma, though local adverse effects, such as hoarseness and oropharyngeal candidiasis, are often seen in patients treated with inhaled corticosteroids. Since the prevalence of candidiasis is positively correlated with increased dose and dosing frequency,[1,2] it is considered that effective prevention against local adverse effects can be achieved by mouth washing after inhalation.[3–6] However, there is no known report regarding an optimal procedure for mouth washing following administration with inhaled budesonide. Recently, we reported the effects of various mouth washing procedures on the removal of drug residues after use of a beclomethasone dipropionate metered dose inhaler (BDP-MDI) and a fluticasone propionate dry powder inhaler (FP-DPI) via a Diskhaler.[7–9] These results suggested that mouth washing was very useful for the removal of corticosteroids following inhalation. Further, we recommended that immediate gargling and rinsing following inhalation with BDP-MDI or FP-DPI be used for the prevention of local adverse effects.

Administration of budesonide (BUD) via a Turbuhaler (BUD-TH) is performed with a dry powder inhaler (DPI) similar to an FP-DPI, though the structure of the devices is different. In addition, a carrier (lactose) is used with FP-DPI, but not with BUD-TH. In the present study, we determined the amounts of drug residues following various mouth washing methods to elucidate their effects on the removal of drugs after use of a BUD-TH.

MATERIALS AND METHODS

Five healthy volunteers, (4 males, 1 female; mean

*e-mail: yamada@ps.toyaku.ac.jp

age 36.2±7.8 years old, range 28–47 years) participated in this study. Patients with asthma that use inhaled corticosteroids range widely in age from children to adults, and local adverse effects from inhaled corticosteroid are common findings in all ages.[5] Thus, the age range of the present volunteers was considered to be appropriate. The objectives and protocol were fully explained to each, and signed informed consent was obtained from all subjects prior to beginning the study.

For the inhaled corticosteroids, we used a Pulmicort 100 Turbuhaler® (AstraZeneca) to administer BUD, which was delivered by the use of a DPI via a TH. As for the BUD-TH, the procedure was performed according to the enclosed Information Leaflet. All subjects were trained to perform the correct procedure of inhalation and mouth washing several times before starting the experiment, and read again the enclosed Information Leaflet just prior to inhalation under observation. The experiments were mouth washing procedure, mouth washing duration, and lag time from inhalation until mouth washing, and were performed in that order. The effects of the various methods of mouth washing on the removal of drug residues from the mouth were evaluated by quantification of the amount of BUD in the expectorated mouth washing rinse after inhalation, using a high-performance liquid chromatographic method.

Statistical differences in the amounts of recovered drugs among the mouth washing methods tested were evaluated by analysis of variance (ANOVA). The duration times of mouth washing were compared statistically used a two-sided *t*-test. These data were analyzed using the Statcel software package (OMS Publishing Inc., Japan). The relationship between the amount of recovered drug and lag time from inhalation until mouth washing was compared statistically by Dunnett's test using the JMP 6. 0. 3 (SAS Institute Inc., Japan). Statistical tests were with the level of significance set at 5%.

Drug Administration The effects of mouth washing on the removal of drug residues were investigated following sprinkling with BUD powder and inhalation with a BUD-TH. A BUD-TH device is unique in that it dispenses minute quantities of BUD powder, without the use of an added carrier such as lactose. We used a diluted BUD powder preparation, as it was difficult to dispense a minute quantity (100 μg) of BUD powder into the mouth. To prepare a 5 μg/mg dose of BUD powder, we used BUD (Sigma, Lot. 81K1654) and lactose (LACTOHALE®).

Mouth Washing Methods Three different types of mouth washing methods were used; gargling and then rinsing the mouth with water for 5 s each, rinsing only for 10 s, and gargling only for 10 s. The total volume of water for each individual mouth washing procedure was 100 ml, which was divided into 5 aliquots of 20 ml each. In a single trial, the mouth washing method was repeated 5 times, with each rinse collected for examination.

Sample Collection, Chromatographic Conditions The collected mouth washing sample was added to 5 ml of chloroform containing *trans*-stilbene (0.1 mg) as an internal standard and the mixture was shaken for 5 min, then centrifuged for 5 min at 3000 rpm, after which the aqueous phase was extracted. The lower organic phase was transferred to a clean conical tube and centrifuged again for 5 min at 3000 rpm. The lower organic phase of 3 ml was then transferred to a clean conical tube and evaporated. The residue was dissolved in 1 ml in the mobile phase and 20 μl was finally injected into a chromatograph.

Chromatographic analyzes were performed using an LC-9A high-performance liquid chromatograph (Shimadzu, Japan) equipped with a variable-volume injector, an automatic sampling system, and an SPD-6A UV detector operating at 250 nm. The separation column was a 5- μm PEGASIL-B ODS column (4.6φ ×250 mm) (Senshu Scientific Co., Ltd., Japan) operating at 40°C. During assay development, BUD was eluted with a mobile phase that consisted of acetonitrile-10 mM potassium dihydrogenphosphate (7 : 1, v/v) at a flow rate of 0.8 ml/min.

Duration of Mouth Washing To determine the effects of mouth washing duration, water was used to gargle and rinse for 3 and 5 s, respectively, and the same sampling method noted above was utilized.

Lag Time from Inhalation until Mouth Washing Lag times of 0, 1, 2.5, 5, and 10 min between inhalation and mouth washing were investigated. For the mouth washing procedure, the subjects were asked to gargle and rinse with water for 5 s each. The total volume of water used for mouth washing was 60 ml, which was divided into 3 aliquots of 20 ml each.[9]

RESULTS

Amounts of Drug Residues after Inhalation with BUD-TH Figure 1 shows the amounts of BUD

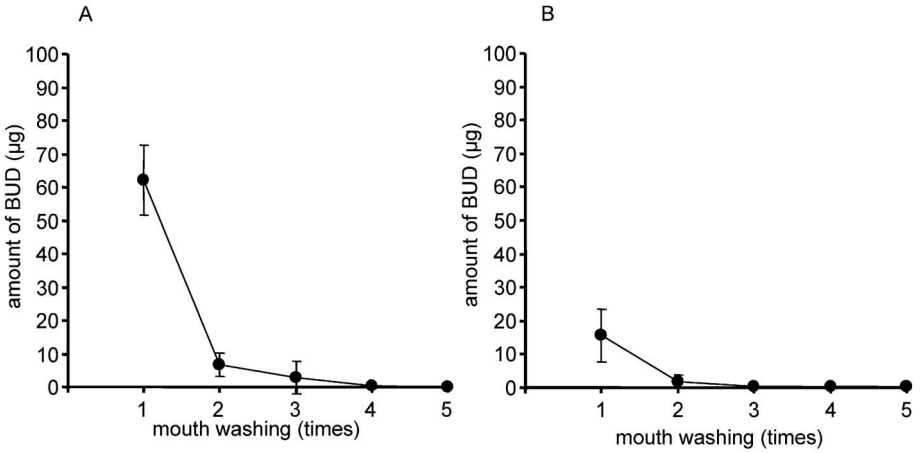

Fig. 1. Amounts of Budesonide Recovered by Gargling and then Rinsing the Mouth with Water for 5 s each after Sprinkling Dry Powder (A) and Inhalation (B)
Results are shown as the means ±S.D. of 5 trials.

recovered following mouth washing after sprinkling with BUD powder (A) and inhalation with the BUD-TH (B). The method of mouth washing was used gargling and rinsing for 5 s each. Total recovered BUD after sprinkling and inhalation were 79.3±2.7 μg and 19.4±9.4 μg, respectively, with a large individual difference observed for the amount of drug recovered. Figure 1(B) shows that the relative ratios of drug residue recovered with BUD-TH were 81.8% by mouth washing once and 92.4% by mouth washing twice, as compared to the recovered amount of drug following mouth washing 5 times.

Effects of Mouth Washing Procedures on Removal of Drug Residues Figure 2 shows the amounts of BUD recovered after performance of the three different methods of mouth washing following use of the BUD-TH. The amount of BUD recovered was with gargling and rinsing for 5 s each (19.4±9.4 μg), rinsing only (23.8±13.6 μg) and gargling only (18.3±8.9 μg). No significant differences were observed among these methods.

Effects of Duration of Mouth Washing on Removal of Drug Residues Figure 3 shows the amounts of drugs recovered following gargling and rinsing for 3 or 5 s following use of the BUD-TH. The amount of BUD was 26.3±8.2 μg after 3 s and 19.4±9.4 μg after 5 s, which were not significantly different. The necessary number of mouth washing for drug residue removed in the mouth was set as the number to achieve a recovery ratio greater than 90%.[7] This number of 'gargling and rinsing' and 'rinsing alone' was twice, respectively. On the other hand, this num-

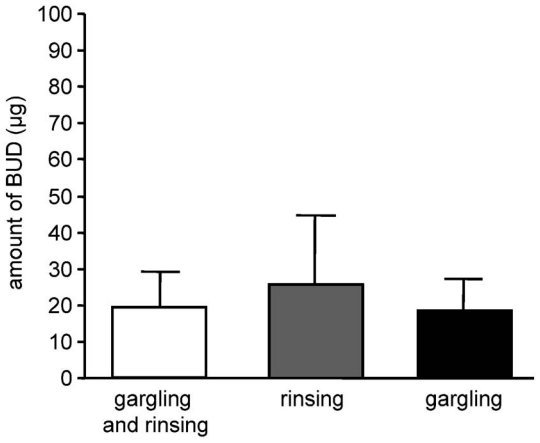

Fig. 2. Amounts of Budesonide Recovered with Various Mouth Washing Procedures Following Inhalation
Results are shown as the means ±S.D. of 5 trials.

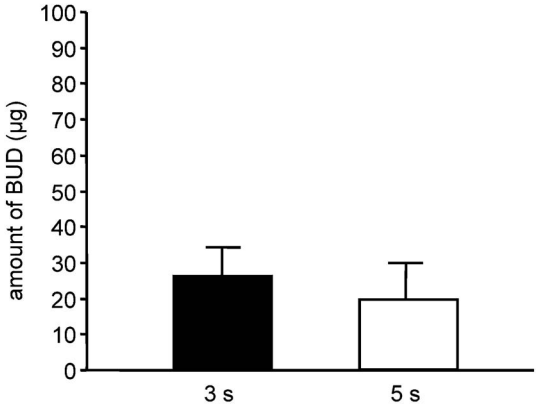

Fig. 3. Amounts of Budesonide Recovered with Different Mouth Washing Durations
Results are shown as the means ±S.D. of 5 trials.

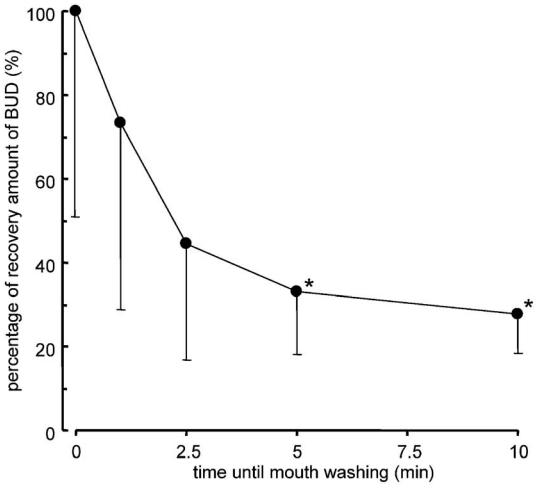

Fig. 4. Amounts of Budesonide Recovered Following Various Lag Times after Inhalation before Mouth Washing

Data are presented as the percentage of amount of drugs recovered by mouth washing compared to immediately after inhalation. Results are shown as the means −S.D. $^{*}p<0.05$ (vs. mouth washing immediately after inhalation).

ber of gargling was three times.

Effects of Lag Time from Inhalation until Mouth Washing on the Removal of Drug Residues Figure 4 shows the relationships between different lag times from inhalation until mouth washing in regard to the amounts of drugs recovered. The percentage of recovery amounts of BUD were 73.2±44.5% after 1 min and 27.8±9.4% after 10 min, as compared to immediately following inhalation (0 min).

DISCUSSION

Local adverse effects, such as oropharyngeal candidiasis and hoarseness, are often seen in patients following treatment with inhaled corticosteroids. As a means of prevention, it is recommended that mouth washing after inhalation is performed, however, there is no known study that compared the effectiveness of different mouth washing methods. We previously reported the effects of different mouth washing procedures on the removal of drug residues after use of a BDP-MDI and an FP-DPI.[7–9] In the present study, we investigated the effects of removal of drug residues using various methods of mouth washing after inhalation with a BUD-TH. The amount of BUD recovered following mouth washing after sprinkling of 100 μg of BUD was 79.3 μg, and mouth washing was effective for recovering of drug residues. The difference in amount of removal drug by mouth washing between sprinkling (79.3%) and inhaling (19.4%) was 59.9 %. In the previous study by another researcher of healthy volunteers who inhaled BUD-TH, drug residue remaining in the device was 22% and lung deposition of drug was 32%.[10] These data were similar to the difference between sprinkling and inhaling in the present study. Thus, we thought that the difference between sprinkling and inhaling was mainly due to drug remaining in the device and drug distributed in the lung after inhalation. The relative removal amount of BUD was 81.8% by mouth washing once and 92.4% by mouth washing twice following inhalation with a BUD-TH, as compared to the recovered amount of BUD after that 5 times. Thus, mouth washing was considered effective to remove drug residues after inhalation with a BUD-TH and that repeated twice was able to achieve a recovery ratio greater than 90%.[7,8] The present results were similar to those found in our previous studies with the BDP-MDI and FP-DPI.[7,8]

No significant differences were observed among the three different methods of mouth washing (gargling and rinsing, rinsing only, gargling only) used for removal of drug residues following inhalation with a BUD-TH. However, we thought that either 'gargling and rinsing' or 'rinsing alone' was better mouth washing procedure, since necessary number of 'gargling and rinsing' and 'rinsing alone' were twice. Nevertheless, additional investigations are necessary to clarify which of methods would be recommended. Our results also suggested that the duration of mouth washing did not have a significant effect on the amount of drug residues removed, which were similar to our results with the BDP-MDI and FP-DPI.[9] However, the individual difference in amount of drug by mouth washing after inhalation with a BUD-TH was significantly greater than that with the BDP-MDI and FP-DPI. As a reason for the discrepancy, we considered that the coefficient of variation (CV) for the dose of BUD adhered to the oropharyngeal area was 30.2%[11] following inhalation with a BUD-TH.

The lag time between inhalation and mouth washing had a significant effect on the amount of drugs removed by mouth washing. In our previous studies, the amounts of drugs removed by mouth washing after inhalation with BDP-MDI and FP-DPI decreased over time.[9] In the present study with BUD-TH, immediate mouth washing after inhalation was considered to be the most effective, as the results with the BUD-TH were nearly the same as with inhalation of

those other corticosteroids. Thus, our findings confirmed that if mouth washing is not performed immediately after inhalation, drug residues are swallowed into the throat along with saliva. The relative recovered amounts of drugs were 73.2±44.5% after 1 min and 27.8±9.4% after 10 min, as compared to immediately following inhalation (0 min). Since the prevalence of candidiasis as a local adverse effect is positively correlated with increased dose and dosing frequency,[1,2] we consider that the prevention against local adverse effect is possible by removing as much of drug residue as possible. Thus, immediate mouth washing after inhalation with a BUD-TH is considered important for the prevention of local adverse effects.

In the present study, mouth washing after inhalation with a BUD-TH was a convenient and effective method for the prevention of local adverse effects, and immediate mouth washing after inhalation was found to be the most effective. We recommend immediate mouth washing after inhalation with a BUD-TH, repeated at least twice. If mouth washing is not performed immediately after inhalation, drug residues will remain and may lead to adverse effects.

REFERENCES

1) Toogood J. H., Jennings B., Baskerville J., Lefcoe N. M., *Eur. J. Respir. Dis.*, **65**, 321–338 (1984).
2) Toogood J. H., Jennings B., Greenway R. W., Chuang L., *J. Allergy Clin. Immunol.*, **65**, 145–153 (1980).
3) Hanania N. A., Chapman K. R., Kesten S., *Am. J. Med.*, **98**, 196–208 (1995).
4) Maxwell D. L., *Biomed. Pharmacother.*, **44**, 421–427 (1990).
5) Ellepola A. N. B., Samaranayake L. P., *Oral Dis.*, **7**, 211–216 (2001).
6) Simon M. R., Houser W. L., Smith K. A., Long P. M., *Ann. Allergy Asthma Immunol.*, **79**, 333–338 (1997).
7) Yamada Y., Hosokawa M., Yamaguchi N., Santa T., Kotaki H., Sawada Y., Iga T., *Yakugaku Zasshi*, **119**, 436–443 (1999).
8) Yokoyama H., Yamada Y., Yamamura Y., Nakamura H., Iga T., *Yakugaku Zasshi*, **121**, 233–237 (2001).
9) Yokoyama H., Yamamura Y., Ozeki T., Iga T., Yamada Y., *Biol. Pharm. Bull.*, **29**, 1923–1925 (2006).
10) O'Connor B. J., *Resp. Med.*, SupA, S10–S16 (2004).
11) Thorsson L., Kenyon C., Newman S. P., Borgstrom L., *Int. J. Pham.*, **168**, 119–127 (1998).